REMARKS

The specification is amended herein to correct typographical errors.

Claims 1 and 4-6 are amended. Claims 11-18 are canceled.

No new matter is presented.

I. Response to Claim Rejection under 35 U.S.C. § 112, 2nd Paragraph

In paragraph 2 of the Office Action, the Examiner has rejected claim 17 under 35 U.S.C. 112, second paragraph.

Claim 17 is canceled herein, thereby rendering the rejection moot.

Accordingly, Applicants respectfully request withdrawal of the rejection.

II. Response to Claim Rejection under 35 U.S.C. § 112, 1st Paragraph

In paragraph 3 of the Office Action, the Examiner has rejected claims 11-18 under 35 U.S.C. §112, first paragraph.

Claims 11-18 are canceled herein, thereby rendering the rejection as to these claims moot.

Accordingly, Applicants respectfully request withdrawal of the rejection.

III. Response to Claim Rejections under 35 U.S.C. § 102

A. Rettenmeier et al

In paragraph 4 of the Office Action, the Examiner has rejected claims 1, 4-6 and 8-16 under 35 U.S.C. §102(b) as allegedly being anticipated by Rettenmeier et al (Drug Metabolism and Disposition, Metabolic Fate of Valproic Acid in the Rhesus Monkey, 1986, 14(4), pages 443-453).

Claims 1 and 6 are amended herein to recite that m in Formula I and Formula I-2, respectively, is an integer of 1-10. Rettenmeier et al does not specifically disclose compounds within the scope of the amended claims 1 and 6 and the claims dependent thereon. Therefore, the present invention is not anticipated.

Claims 11-16 are canceled, thereby rendering the rejection moot as to these claims.

Accordingly, Applicants respectfully request withdrawal of the rejection.

B. Ohuchida et al (US '021)

In paragraph 5 of the Office Action, the Examiner has rejected claims 1, 9, 11-15 and 19 under 35 U.S.C. §102(b) as allegedly being anticipated by Ohuchida et al (US 6,201,021).

Claims 1, 4, and 5 are amended herein as follows:

(a) the definition of R^1 in claim 1 is changed from "optionally protected hydroxy or oxo" to --hydroxy or oxo--;

(b) the definition of R^{1-1} and R^{1-2} in claims 4 and 6 is changed from "a hydrogen atom or optionally protected hydroxy" to --a hydrogen atom or hydroxy--;

(c) the definition of R^{1-3} in claims 4 and 6 is changed from "a hydrogen atom or optionally protected hydroxy" to --a hydrogen atom or hydroxy--;

(d) the definition of R^{1-4} and R^{1-5} in claim 5 is changed from "a hydrogen atom or optionally protected hydroxy" to --a hydrogen atom or hydroxy--; and

(e) the definition of R^{1-6} in claim 5 is changed from "a hydrogen atom or optionally protected hydroxy" to --a hydrogen atom or hydroxy--.

Ohuchida et al does not specifically disclose compounds within the scope of the amended claims 1, 4 and 5 and claims dependent thereon. Therefore, the present invention is not anticipated.

Claims 11-15 are canceled herein, thereby rendering the rejection as these claims moot.

Accordingly, Applicants respectfully request withdrawal of the rejection.

IV. Response to Claim Rejections under 35 U.S.C. § 103

In paragraph 6 of the Office Action, the Examiner has rejected claims 1-16 and 19 under 35 U.S.C. §103(a) as allegedly being unpatentable over by Ohuchida et al (US 6,201,021 03-2001) in view of Rettenmeier et al (Drug Metabolism and Disposition, Metabolic Fate of Valproic Acid in the Rhesus Monkey, 1986, 14(4), pages 443-453).

Applicants respectfully traverse the rejection.

As pointed out by the Examiner, Ohuchida et al describes 5-methoxy-2-propylpentanoic acid and 6-methoxy-2-propylhexanoic acid which can be used for treating diseases due to reactive astrocytes. Also, as pointed out by the Examiner, Rettenmeier et al describes several valproic acid metabolites. However, Applicants submit that one of ordinary skill in the art would not have been motivated to combine the references with a reasonable expectation of success in achieving the present invention.

Generally, drug metabolism is a mechanism for improving the water solubility of a compound administered into the living body and then facilitating its excretion. The compound after metabolism usually loses its pharmacological activity, except for a compound which is synthesized so as to exert its activity after metabolism, such as a prodrug. In Rettenmeier et al, in order to clarify substances which cause hepatic injury which occurrs by use of valproic acid in rare cases, valproic acid is administered to rhesus monkeys to examine the metabolites produced. The metabolites are hydroxy-VPA such as 3-OH-VPA, 4-OH-VPA and 5-OH-VPA and oxo VPA such as 3-oxo-VPA and 4-oxo-VPA (valproic acid metabolites 9-12 and 15 pointed out by

the Examiner). There are many studies for metabolites of valproic acid before Rettenmeier et al. For example, Table 1 in *Neuropharmacology*, 24, 427-435 (1985) (Attachment A) shows that the pharmacological activity of valproic acid disappears or is remarkably decreased by metabolism into hydroxy-VPA. Thus, one of ordinary skill in the art would not have been motivated to combine the references with a reasonable expectation of success.

Even if Ohuchida et al and Rettenmeier et al were combined, one of ordinary skill in the art would consider only that the pharmacological activity of a branched chain aliphatic acid which is useful for treating diseases due to reactive astrocytes is maintained by methoxylation of its aliphatic acid part, but is not maintained by hydroxylation or oxolation. One of ordinary skill in the art would not have been motivated to hydroxylate or oxolate an aliphatic acid compound in order to obtain a compound useful for treating diseases due to reactive astrocytes.

Furthermore, methoxy-VPA is not within the scope of the present claims.

In view of the above, even if Ohuchida et al and Rettenmeier et al are combined, the present invention cannot easily be expected by one of ordinary skill in the art.

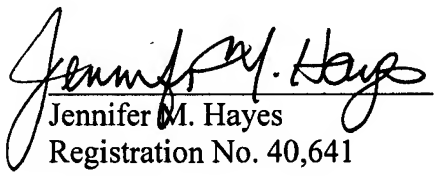
Accordingly, Applicants respectfully request withdrawal of the rejection.

V. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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PHARMACOLOGICAL EVALUATION OF VARIOUS METABOLITES AND ANALOGUES OF VALPROIC ACID

ANTICONVULSANT AND TOXIC POTENCIES IN MICE

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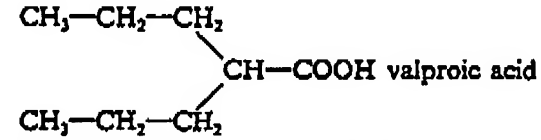
(*Accepted* 17 *September* 1984)

Summary—Thirty-two metabolites and analogues of the antiepileptic drug valproic acid (2-propylpentanoic acid; VPA) were tested for anticonvulsant and toxic effects in mice, in an attempt to find out if any of these compounds were superior to valproic acid. Valproic acid and ethosuximide, another clinically established antiepileptic drug, were included in these studies for comparison. After intraperitoneal administration, the anticonvulsant potency of the various drugs was determined in three seizure tests: the threshold for maximal electroconvulsions, the maximal electroshock seizure test and seizures induced by subcutaneous injection of pentylenetetrazol. For the most potent compounds, median minimal neurotoxic doses (TD_{50}s) and LD_{50}s (after i.p. and i.v. injection) were determined. Valpramide, the primary amide of valproic acid, proved to be the most potent compound in the three seizure tests, used, being 2–5 times as potent as valproic acid, but valpramide was also considerably more sedative and toxic than valproic acid or ethosuximide. Of the metabolites of valproic acid tested, the unsaturated compounds 4-en-valproic acid (4-en-VPA) and the *trans*-isomer of 2-en-valproic acid (2-en-VPA) were most potent and, depending on the seizure test used, reached 60–100% of the efficacy of the parent drug. Both metabolites had LD_{50} values which were similar or greater than those of valproic acid but they were more sedative than the parent compound. Analogues of valproic acid with shorter side-chain lengths were only weakly active as anticonvulsants, whereas elongation of the side-chains led to increases in anticonvulsant potency but also in sedative/hypnotic side effects and toxicity. Non-branched monocarboxylic acids and cyclic compounds in which the side-chains have been closed to a ring were inactive or only weakly active. However, addition of a methyl group in position 1 at the ring of cyclohexanoic acid markedly increased the anticonvulsant potency without altering LD_{50} values. Similarly, introduction of an additional branching with a methyl group at C2 of analogues of valproic acid led to considerable enhancement of anticonvulsant effectiveness. Although these methyl-substituted compounds were more sedative than valproic acid, they seem to be interesting tools with regard to the structural prerequisites of anticonvulsant, toxic and teratogenic effects of branched fatty acids.

Key words: valproic acid, metabolites, analogues, anticonvulsant screening, toxicity, mice.

Current available antiepileptic drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvement, whereas the remainder does not benefit significantly (Coatsworth, 1971; O'Leary and Goldring, 1976). Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult. Thus, notwithstanding the beneficial effects of the current drugs, there is still a demand for new antiepileptic drugs with more selective anticonvulsant effects and less toxicity.

There are different approaches to the search for new antiepileptic drugs (cf. Krall, Penry, White, Kupferberg and Swinyard, 1978). The most common is the synthesis of derivatives of already known and active drugs. As regards the widely used antiepileptic drug valproic acid (VPA; 2-propylpentanoic acid, dipropylacetic acid; see structure below) numerous derivatives of this drug



have been tested for anticonvulsant action (Carraz, Bériel, Luu-Doc and Lebreton, 1965; Benoit-Guyot, Benoit-Guyot, Boucherle, Broll and Eymard, 1972a, b, 1973; Lespagnol, Mercier, Erb-Debruyne and Dessaigne, 1972; Maitre, Ciesielski and Mandel, 1974; Taillandier, Benoit-Guyot, Boucherle, Broll and Eymard, 1975; Whittle and Turner, 1981; Chapman, Meldrum and Mendes, 1983; Keane, Simiand, Mendes, Santucci and Morre, 1983). Furthermore, various metabolites of valproic acid have been found to exert anticonvulsant activity in rodents (Taillandier *et al.*, 1975; Weissman, Simler, Ciesielski and Mandel, 1978; Schäfer, Lührs and Reith, 1980; Löscher, 1981). However, although almost 200 com-

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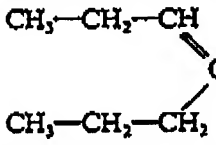
pounds have been tested so far, no systematic study is available in which anticonvulsant and toxic potencies of analogues and metabolites of valproic acid have been quantified and compared in standard animal models for screening of anticonvulsant drugs (cf. Krall *et al.*, 1978) in an attempt to find out if any of these compounds may be superior to valproic acid.

During the last 5 years, such a systematic study has been embarked on. As a first promising result, it was recently reported that 2-en-valproic acid (2-propylpentenoic acid; see structure below), the major active metabolite of valproic acid is as



2-en-valproic acid

potent as the drug in different animal models of epilepsy, but lacks the serious embryotoxic effects of valproic acid, at least in a rodent model (Löscher, Nau, Marescaux and Vergnes, 1984; Nau, Löscher and Schäfer, 1984). The anticonvulsant and toxic potencies of various other metabolites and analogues of valproic acid in mice, including several compounds that have not been tested before are now reported. Valproic acid and another clinically established antiepileptic drug, i.e. ethosuximide, were used as reference standards in this study.

METHODS

Animals

Male mice of the NMRI strain (Charles River Wiga GmbH, Sulzfeld, FRG), weighing 25–30 g, were used. All experiments were performed at an ambient temperature of 25°C and controlled humidity (approx. 50%) during the forenoon.

Determination of anticonvulsant potencies

As a first screen, the effect of valproic acid and various metabolites and analogues of valproic acid on the threshold for maximal electroconvulsions was determined. All compounds were injected intraperitoneally in groups of 20 mice at doses of 75–200 mg/kg and threshold determinations were made at 30 min after injection. The electroshock was delivered via eye electrodes for 0.2 sec (50 cycles/sec) from a Lafayette A-625 B shocker (Lafayette Instrument Co., Indiana, U.S.A.) with the serial resistance set to 10 kOhm. The extension of the hind limbs was taken as the endpoint. The threshold was determined in volts by the "up and down" method of Kimball, Burnett and Doherty (1957) and calculated as the voltage inducing the extensor phase in 50% of the mice (EV_{50}). All treated groups were compared with concurrent control groups. The EV_{50} of controls was between 130 and 140 V. On each experimental day, a group of mice was treated with valproic acid for comparison of potency with the test drugs. Anticonvulsant potency relative to valproic acid, on molar base was calculated for test compounds as follows: the increase in voltage above the control EV_{50} caused by the test drug was divided by the increase in voltage induced by valproic acid. This ratio was then multiplied by the ratio between the molar dose of valproic acid and the molar dose of the test compound. Only significant increases in threshold (P at least < 0.05 by Student's t-test) were used for calculation of potencies.

Drugs which showed at least 80% of the anticonvulsant potency of valproic acid in this first screen were selected for further evaluation in the tests described below. Ethosuximide was included in these tests for comparison.

The maximal electroshock seizure (MES) test was performed by means of the Lafayette shocker as described above except that the current was set constantly to 250 V, which was about twice the threshold for tonic electroshock seizures. Tonic extension of the hind limbs was used as the endpoint. Dose–effect curves were constructed for each drug by using 10 mice per dose.

In further groups of 10 mice, anticonvulsant potency against seizures induced by pentylenetetrazol (100 mg/kg, s.c.) was determined 0.5 hr after intraperitoneal injection of the drugs studied. Mice which did not show generalized clonic or tonic seizures with loss of the righting reflexes within 30 min after injection of pentylenetetrazol were considered as protected. Calculation of ED_{50} values against maximal electroshock and pentylenetetrazol was done by the method of Litchfield and Wilcoxon (1949).

Toxicity

The median minimal neurotoxic dose (TD_{50}) was determined 0.5 hr after intraperitoneal injection of the different compounds by the chimney test of Boissier, Tardy and Diverres (1960). This test has been designed to detect minimal neurological deficit (e.g. impairment of motor coordination, sedation, catalepsy) and is in this respect similar to the rotorod test of Dunham and Miya (1957). Actually, for some of the compounds studied here the minimal neurotoxicity was determined in both tests and found essentially to reveal the same TD_{50} values. In the chimney test, neurological deficit is indicated by the inability of the mice to climb up backwards in a glass tube of 25 cm length and 3 cm inner diameter within 30 sec. Normal mice climb up in 5–10 sec.

The acute LD_{50} was determined after intraperitoneal and intravenous administration. The mice were observed for 14 days for delayed deaths. Calculation of TD_{50} and LD_{50} values was done by the method of Litchfield and Wilcoxon (1949) using groups of 10 mice per dose.

Drugs used

All metabolites and analogues of valproic acid used in this study (see Table 1) were kindly provided by Dr

Table 1. Estimate of the anticonvulsant potency of various metabolites and analogues of valproic acid (VPA) in mice as determined by their effect on the threshold for maximal electroconvulsions

Drugs	Relative anticonvulsant potency on molar base	Selected for further evaluation
Valproic acid (VPA; 2-propylpentanoic acid)	1.0	
Valpramide (VPA-amide)	5.2	+
VPA-metabolites		
2-Propyl-3-hydroxy-pentanoic acid (3-hydroxy-VPA)	0.13	
2-Propyl-4-hydroxy-pentanoic acid (4-hydroxy-VPA)	0.26	
2-Propyl-5-hydroxy-pentanoic acid (5-hydroxy-VPA)	0	
2-Propyl-3-keto-pentanoic acid (3-keto-VPA)	0.17	
2-Propyl-4-pentenoic acid (4-en-VPA)	0.87	+
2-Propyl-3-pentenoic acid (3-en-VPA)	0.54	
2-Propyl-2-pentenoic acid (2-en-VPA)		
cis-isomer	0.56	
trans-isomer	0.84	+
2-(Propenyl-4)-2-pentenoic acid (4,2'-dien-VPA)	0.46	
2-(Propenyl-4)-4-pentenoic acid (4,4'-dien-VPA)	0.29	
2-*n*-Propylglutaric acid	0.11	
VPA-analogues		
2-Ethylbutyric acid	0.22	
2-Methylpentanoic acid	0.18	
2-Ethylpentanoic acid	0.25	
2-Methylhexanoic acid	0	
2-Ethylhexanoic acid	0.4	
2-Propylhexanoic acid	0.96	+
2-Butylhexanoic acid (dibutylacetic acid)	1.6	+
2-Pentylheptanoic acid	1.4	+
2-Hexyloctanoic acid	3.0	+
3-Propylhexanoic acid	3.7	+
2-Butyl-3-methylpentanoic acid	0.82	
2-Isopropyl-3-methylbutyric acid (di-isopropyylacetic acid)	0.58	
2,2-Dimethylpentanoic acid (2,2-dimethylvaleric acid)	1.6	+
2-Ethyl-2-methylpentanoic acid (2-ethyl-2-methylcaproic acid)	2.7	+
Octanoic acid (caprylic acid)	0	
4-Pentenoic acid	0	
Cyclopentanoic acid	0	
Cyclohexanoic acid	0.11	
Cycloheptanoic acid	0.21	
1-Methyl-1-cyclohexanoic acid	0.88	+

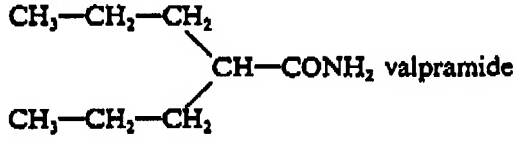
All compounds were administered intraperitoneally and threshold determination were made 0.5 hr after injection. Anticonvulsant potency relative to valproic acid(= 1) was calculated on a molar base by the increase in seizure threshold as described in the Methods section. Compounds with a relative anticonvulsant potency of at least 0.8 were selected for further evaluation.

H. Schäfer (Desitin-Werk Carl Klinke, Hamburg, FRG) and Professor W. Kochen (Children's Hospital, University of Heidelberg, FRG) except the commercially-available compounds 2-*n*-propylglutaric acid (Ferak, Berlin), 2-ethyl-2-methylcaproic acid and 4-pentenoic acid (Fluka AG, Ulm, FRG), 2,2-dimethylvaleric acid (ICN Pharmaceuticals, Plainview, New York, U.S.A.), octanoic acid (E. Merck, Darmstadt, FRG), and the cyclic valproic acid derivatives (EGA-Chemie, Steinheim, FRG). Valproic acid, used as its sodium salt, and ethosuximide were gifts from Desitin-Werk Carl Klinke (Hamburg, FRG). All drugs, except valpramide, were freshly dissolved in distilled water, the free acids by means of equimolar amounts of NaOH and administered intraperitoneally in a volume of 10 ml/kg. Valpramide, the amide of valproic acid, was suspended in distilled water containing 1% Tween 80 by means of a Potter Elvejhem glass homogenizer with a motor-driven Teflon pestle. In all cases the doses of compounds given in this study refer to the amount of free acid (or amide in the case of valpramide).

RESULTS

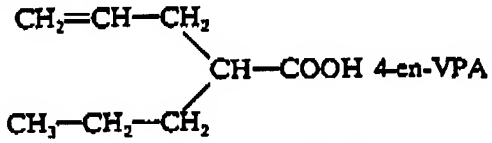
Anticonvulsant potency of metabolites and analogues of valproic acid in seizure threshold experiments

As a first screen for anticonvulsant action, the effect of 32 metabolites and analogues of valproic acid on the threshold for maximal electroconvulsions was determined and compared with the effect of the parent drug. The anticonvulsant potencies on molar base relative to valproic acid thus obtained are shown in Table 1. Valpramide, the primary amide of valproic acid (see structure below), proved to be the most potent compound in this test-system, being about five times more active than valproic acid.



With respect to metabolites of valproic acid, all compounds except 5-hydroxy-valproic acid significantly increased the electroconvulsive thresh-

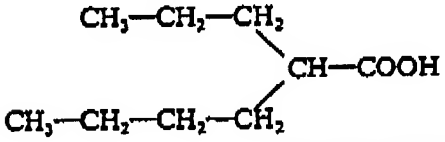
old, but none reached the activity of the parent drug. The greatest potencies were displayed by the unsaturated compounds 4-en-valproic acid (4-en-VPA) (see structure below) and the *trans*-isomer of 2-en-valproic acid (2-en-VPA) (see structure in the introduction), which possessed 84–87% of the activity of valproic acid.



4-en-VPA

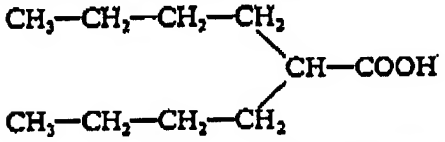
The *cis*-isomer of 2-en-valproic acid, 3-en-valproic acid and the double unsaturated metabolites 4,2′-dien-valproic acid and 4,4′-dien-valproic acid were considerably less potent. Similary the hydroxylated metabolites, 3-keto-valproic acid, and 2-*n*-propylglutaric acid were only weakly active.

Among close analogues of valproic acid with branching in the 2-position, compounds in which one or both side-chains were shorter compared to valproic acid, namely 2-ethylbutyric acid, 2-methylpentanoic acid, 2-ethylpentanoic acid, 2-methylhexanoic acid and 2-ethylhexanoic acid, were considerably less active than valproic acid. Elongation of one side-chain resulted in no increase of anticonvulsive potency compared to valproic acid, as shown by 2-propylhexanoic acid. On the other hand, elongation of both



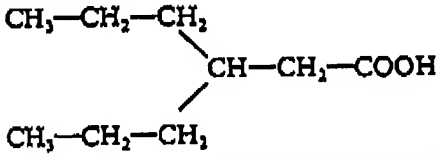
2-propylhexanoic acid

side-chains increased anticonvulsant activity progressively as shown by the relative potencies of 2-butylhexanoic acid (see structure below), 2-pentylhexanoic acid and 2-hexyloctanoic acid.



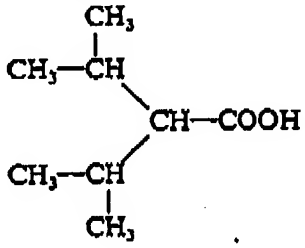
2-butylhexanoic acid

A considerable enhancement of anticonvulsant potency could be reached by branching in position 3 instead of 2. Thus, 3-propylhexanoic acid was about 4 times more potent than 2-propylhexanoic acid and valproic acid.



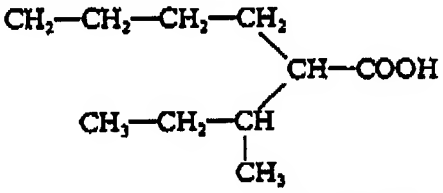
3-propylhexanoic acid

Introduction of additional branchings with methyl groups at C3 only moderately influenced anticonvulsant activity. Thus, 2-isopropyl-3-methylbutyric acid (di-isopropylacetic acid; see structure below) was somewhat more active than



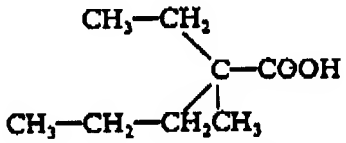
di-isopropylacetic acid

the corresponding compound without the 2 methyl groups (2-ethylbutyric acid), but was still considerably less potent than valproic acid, although the number of carbons in the molecule was the same. 2-Butyl-3-methylpentanoic acid was even less active than the corresponding compound with 2 carbon chains (2-propylhexanoic acid).



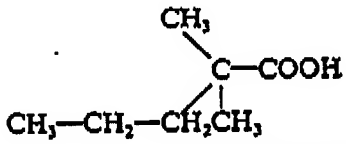
2-butyl-3-methylpentanoic acid

On the other hand, additional branching with methyl groups at C2 considerably enhanced the anticonvulsant activity. Thus, 2-ethyl-2-methylpentanoic acid (2-ethyl-2-methylcaproic acid; see structure below) was 10 times more potent



2-ethyl-2-methylpentanoic acid

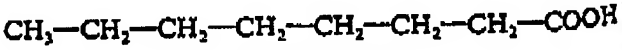
than 2-ethylpentanoic acid, and about 3 times more potent than valproic acid. Similarly, 2,2-dimethylpentanoic acid (2,2-dimethylvaleric acid) was 9 times more potent



2,2-dimethylpentanoic acid

than the corresponding 2-methylpentanoic acid, and 1.6 times more potent than valproic acid.

Non-branched fatty acids, i.e. octanoic acid and the unsaturated compound 4-pentenoic acid (see structures below), possessed no anticonvulsant activity.

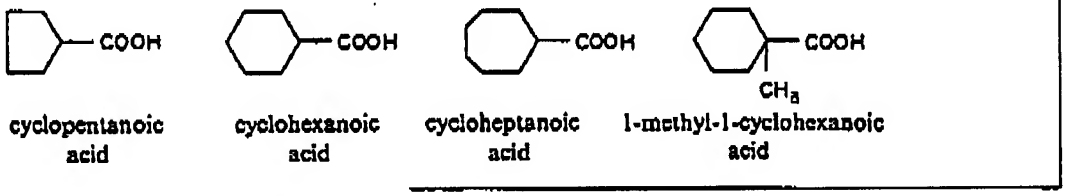


octanoic acid

$CH_2{=}CH{-}CH_2{-}CH_2{-}COOH$

4-pentenoic acid

Cyclic analogues of valproic acid, i.e. cyclopentanoic acid, cyclohexanoic acid and cycloheptanoic acid, were also inactive or only weakly active, However, addition of a methyl group in position 1 at the ring, i.e. 1-methyl-1-cyclohexanoic acid, resulted in a pronounced enhancement of anticonvulsant activity compared to cyclohexanoic acid.



cyclopentanoic acid | cyclohexanoic acid | cycloheptanoic acid | 1-methyl-1-cyclohexanoic acid

Based on the seizure threshold experiments, compounds with a relative anticonvulsant potency of at least 0.8 were selected for further evaluation of anticonvulsant and toxic activities in standard animal models for screening of anticonvulsant drugs. After initial determinations of toxicity, some of these compounds were not studied further because of high toxicity, namely 2-pentylheptanoic acid (LD_{50} 140 mg/kg, i.p.), 2-hexyloctanoic acid (LD_{50} 180 mg/kg, i.p.) and 3-propylhexanoic acid (LD_{50} 430 mg/kg, i.p.). 2-Butyl-3-methylpentanoic acid could not be evaluated in more detail because of the limited amount of substance available.

Anticonvulsant potency of metabolites and analogues of valproic acid in traditional models of epilepsy in the mouse

The 8 compounds finally selected for further assessment of pharmacological effects (see Table 2) were tested in the classical models of epilepsy in the mouse, namely the maximal electroshock seizure (MES) test and seizures induced by subcutaneous injection of pentylenetetrazol. Whereas the maximal electroshock test is the traditional model that predict drugs to be effective against generalized tonic–clonic (*grand mal*) seizures, pentylenetetrazol-induced seizures are considered as a valuable model to predict antiepileptic activity against generalized minor (*petit mal*) seizures in man. Besides the 8 analogues and metabolites of valproic acid, activity of valproic acid itself and of ethosuximide was determined in these models for comparison. Anticonvulsant ED_{50} values as well as potencies relative to valproic acid are shown in Table 2. In general, all compounds except ethosuximide were about equally active against maximal electroshock and pentylenetetrazol-induced seizures.

In the maximal electroshock test, valpramide proved again to be the most potent compound with an ED_{50} of 100 mg/kg, which was about two times less than the respective ED_{50} of valproic acid (210 mg/kg). 2-Ethyl-2-methylpentanoic acid and 2-propylhexanoic acid were 20–30% more active than valproic acid on a molar base, which was significant ($P < 0.05$) when calculated by the method of Litchfield and Wilcoxon (1949). 2-En-valproic acid (*trans*-isomer), 1-methyl-1-cyclohexanoic acid and 2-butylhexanoic acid did not differ significantly in potency from valproic acid, whereas 2,2-dimethylpentanoic acid and 4-en-valproic acid were significantly less potent than the parent drug. The least potent drug was ethosuximide, which protected only 30% of the animals against maximal electroshock seizures at 1000 mg/kg.

In regard to seizures induced by pentylenetetrazol, valpramide was again the most effective drug with 2 times the potency of valproic acid. Ethosuximide was also significantly more potent than valproic acid. Among the remaining 7 compounds, 2-ethyl-2-methylpentanoic acid was the most potent with a relative molar potency of 1.33 (significantly different from valproic acid with $P < 0.05$). All other drugs did not differ significantly in potency from valproic acid, except 4-en-valproic acid which was the least potent drug in this series with a relative potency of 0.72.

Side effects and toxicity of metabolites and analogues of valproic acid

In general, all drugs studied, including valproic acid and ethosuximide, displayed sedative/hypnotic side effects. However, the doses at which these side effects occurred and the extent of the side effects varied considerably among the drugs. In order to quantify the occurrence of these side effects, the median minimal neurotoxic dose (TD_{50}) was determined for each compound by the chimney test at the time of anticonvulsant testing, i.e. 0.5 hr after administration. As shown in Table 2, the lowest TD_{50} values were determined for valpramide and the compounds with methyl substitutions, i.e. 2-ethyl-2-methylpentanoic acid, 1-methyl-1-cyclohexanoic acid and 2-ethyl-2-methylpentanoic acid. Ethosuximide displayed by far the highest TD_{50} value, followed by valproic acid. At these median minimal neurotoxic doses, the only obvious side effects for most drugs were sedation and muscular weakness. However, with several metabolites and analogues of valproic acid moderate increases of doses above the TD_{50} gave rise to much stronger adverse effects, of such as reduced or impaired locomotor activity, impaired righting reflexes and ataxia. This was especially marked in the

Table 2. Anticonvulsant and toxic potencies of valproic acid (VPA), ethosuximide and various metabolites and analogues of valproic acid in mice

Drugs	ED_{50} (mg/kg, i.p.)		Relative potency on molar base (VPA = 1)		TD_{50} (mg/kg, i.p.)	LD_{50} (mg/kg, i.p.)	LD_{50} (mg/kg, i.v.)
	MES-test	PTZ-test	MES	PTZ			
Valproic acid (VPA)	210 (180–240)*	240 (210–280)	1.0	1.0	350 (300–400)	870 (820–910)	630 (530–710)
Ethosuximide	> 1000†	150 (110–200)	< 0.2	1.57	470 (360–610)	> 2000‡	780 (650–940)
Valpramide (VPA-amide)	100 (80–130)	120 (110–130)	2.1	2.0	130 (120–150)	430 (340–540)	n.d.§
2-en-VPA (*trans* isomer)	220 (190–260)	230 (200–260)	0.94	1.03	220 (200–250)	760 (700–830)	1020 (940–1100)
4-en-VPA	350 (330–380)	330 (280–390)	0.59	0.72	360 (330–390)	1160 (1030–1310)	1000 (920–1100)
2-Propylhexanoic acid	190 (180–200)	250 (230–270)	1.21	1.05	200 (180–230)	680 (620–740)	380 (340–430)
2-Butylhexanoic acid (dibutyl-acetic acid)	220 (210–230)	250 (230–280)	1.14	1.15	190 (170–220)	340 (300–390)	170 (150–190)
1-Methyl-1-cyclo-hexanoic acid	230 (210–250)	270 (230–310)	0.90	0.88	180 (130–240)	860 (800–920)	840 (700–1010)
2-Ethyl-2-methyl pentanoic acid	160 (150–170)	180 (160–200)	1.31	1.33	140 (120–160)	390 (350–430)	280 (260–310)
2,2-Dimethyl pentanoic acid	230 (210–250)	190 (180–210)	0.82	1.14	200 (150–260)	900 (840–960)	740 (670–820)

*Confidence limits for 95% probability; †30% protected at this dose;
‡40% died at this dose; §could not be determined because of insolubility in water.
All tests were performed 0.5 hr after administration of the drugs. MES-test refers to the maximal electroshock seizure test, PTZ-test refers to seizures induced by subcutaneous injection of 100 mg/kg pentylenetetrazol. The median minimal neurotoxic dose (TD_{50}) was determined by the chimney test of Boisser *et al.* (1960).

case of valpramide, 2-ethyl-2-methylpentanoic acid and 2-en-valproic acid, which induced such pronounced side effects at doses of 200–300 mg/kg.

With respect to toxicity, LD_{50} values were determined after both intraperitoneal and intravenous administration (Table 2). After intraperitoneal injection, the least toxic compounds were ethosuximide, valproic acid, 2,2-dimethylpentanoic acid and 4-en-valproic acid. 1-Methyl-1-cyclohexanoic acid and 2-en-valproic acid displayed LD_{50} values which were similar to those of valproic acid, whereas 2-butylhexanoic acid, 2-ethyl-2-methylpentanoic acid and valpramide were 2–3 times more toxic. The profile of toxicity after intravenous administration differed from that found after intraperitoneal in that 2-en-valproic acid and 4-en-valproic acid were clearly less toxic than valproic acid and ethosuximide. Again, 2-butylhexanoic acid and 2-ethyl-2-methylpentanoic acid were the most toxic compounds of this series. At toxic doses, all drugs caused marked sedation, ataxia and loss of righting reflexes which occurred instantaneously (after i.v.) or within 1–3 min (after i.p.). Following some compounds, especially 2-en-valproic acid and 2-ethyl-2-methylpentanoic acid, the animals looked as if they had received a general anesthetic and lay unconsciously on their sides for hours. Ethosuximide differed in its side effects from valproic acid and related compounds in that in addition to the sedative/hypnotic symptoms a marked vasodilatation (as indicated by intense reddening of the skin) and stereotyped scratching occurred. Following administration of some compounds, e.g. 2,2-dimethylpentanoic acid and 2-ethyl-2-methylpentanoic acid, delayed deaths occurred after several days.

In order to estimate the margin between anticonvulsant effects and the occurrence of neurotoxic side effects, protective indices were determined by calculating the ratio between the respective TD_{50} values and the anticonvulsant ED_{50} values. A protective index of > 1 would mean that the compound was effective by the maximal electroshock or pentylenetetrazol-induced seizure test in nontoxic doses, whereas an index of $\leqslant 1$ would mean that the drug was only active at neurotoxic doses. As shown in Table 3, the greatest protective indices were found for ethosuximide by the pentylenetetrazol-induced seizure test (3.1) and valproic acid by both seizure tests (1.5–1.7), whereas almost all other compounds had indices of 1–1.1 or less than 1. 2-Butylhexanoic acid and 1-methyl-1-cyclohexanoic acid displayed the smallest protective indices of all compounds studied.

Margins of safety, i.e. the ratio between LD_{50} and anticonvulsant ED_{50} after intraperitoneal administration, are also shown in Table 3. Again, ethosuximide showed the most favourable ratio by the pentylenetetrazol-induced seizure test, whereas 2-butylhexanoic acid and 2-ethyl-2-methylpentanoic acid had the smallest values by both seizure tests. Most other analogues or metabolites of valproic acid had margins of safety very similar to those of valproic acid, i.e. between 3 and 4.

DISCUSSION

For more than 10 years, valproic acid seemed to be

Table 3. Protective indices (TD_{50}/ED_{50}) and margins of safety (LD_{50}/ED_{50}) of valproic acid (VPA), ethosuximide and various metabolites and analogues of valproic acid

Drugs	$\frac{TD_{50}}{ED_{50}}$ (MES)	$\frac{TD_{50}}{ED_{50}}$ (PTZ)	$\frac{LD_{50}}{ED_{50}}$ (MES)	$\frac{LD_{50}}{ED_{50}}$ (PTZ)
Valproic acid (VPA)	1.7	1.5	4.1	3.6
Ethosuximide	< 0.5	3.1	*	> 13
Valpramide	1.3	1.1	4.3	3.6
2-en-VPA	1.0	0.96	3.5	3.3
4-en-VPA	1.0	1.1	3.3	3.5
2-Propylhexanoic acid	1.1	0.8	3.6	2.7
2-Butylhexanoic acid	0.86	0.76	1.5	1.4
1-Methyl-1-cyclohexanoic acid	0.78	0.67	3.7	3.2
2-Ethyl-2-methylpentanoic acid	0.88	0.78	2.4	2.2
2,2-Dimethylpentanoic acid	0.87	1.1	3.9	4.7

*Could not be calculated (see Table 2).
All ratios were determined from the intraperitoneal data shown in Table 2.

an ideal antiepileptic drug in that it proved active against diverse types of epilepsy and, until recently, was thought to be relatively free from serious adverse effects (cf. Pinder, Brogden, Speight and Avery, 1977). However, over the past few years evidence accumulated that therapy in man with valproic acid might be associated with two rare but extremely serious adverse effects, namely hepatic failure resulting in fatalities and teratogenicity, expecially severe neural tube defects (cf. Jeavons, 1984). Hepatotoxic and teratogenic effects of valproic acid have also been reported to occur in experimental animals (cf. Nau and Löscher, 1984). Thus, the search for alternative antiepileptic drugs possessing the desirable properties of valproic acid (low neurotoxicity, wide spectrum of antiepileptic activity) but lacking the hepatotoxic and teratogenic risks became a matter of high priority. As one possible step towards this goal a study of how the pharmacological and toxicological properties of valproic acid were altered by modifications in the chemical structure of the drug was started. It was found that introduction of a double bond between C2 and C3 resulted in a compound (2-en-valproic acid, see structure in the introduction) which as *trans*-isomer is as active as valproic acid in experimental models of epilepsy but lacked the teratogenic effects of the parent drug (Löscher *et al.*, 1984; Nau *et al.*, 1984). Furthermore, studies on concentrations in the liver of 2-en-valproic acid and enzyme activity in the liver following treatment with 2-en-valproic acid in mice and rats suggested that this drug may also be superior to valproic acid with regard to liver toxicity (Löscher and Nau, 1984; Schäfer and Lührs, 1984). Although 2-en-valproic acid is considerably more sedative than valproic acid in mice (also shown in the present study), experiments with 2-en-valproic acid in other species (gerbils with reflex epilepsy and rats with spontaneously occurring spike-wave absence seizures) have shown that this drug is an effective anticonvulsant in small, non-sedative doses (Löscher *et al.*, 1984). Interestingly, 2-en-valproic acid is the major metabolite of valproic acid in various species including man, but its share in the anticonvulsant efficacy of valproic acid during therapy is not clear yet (cf. Nau and Löscher, 1984).

In the present study, the anticonvulsant and toxic properties of various other metabolites and analogues of valproic acid were determined in mice. Studies on the teratogenicity of these compounds will be reported elsewhere. As already shown in part previously (Löscher, 1981), among the metabolites of valproic acid 4-en-valproic acid and the *trans*-isomer of 2-en-valproic acid were the most potent compounds to increase the electroconvulsive threshold, possessing 80–90% of the activity of the parent drug. However, in more traditional models in the mouse for screening of anticonvulsant drugs with (supra)maximal seizure induction, namely the maximal electroshock and pentylenetetrazol induced seizure tests, 4-en-valproic acid was considerably less active than valproic acid and 2-en-valproic acid. Teratological studies in mice have shown that 4-en-valproic acid is teratogenic as valproic acid (Nau and Löscher, 1984). Furthermore, in rats 4-en-valproic acid seems to be markedly more hepatotoxic than valproic acid and 2-en-valproic acid (Schäfer and Lührs, 1984). Thus, in contrast to 2-en-valproic acid, 4-en-valproic acid is certainly not a valuable alternative to valproic acid. Interestingly, by introduction of a second double-bond (4,2'-dien-valproic acid and 4,4'-dien-valproic acid) the anticonvulsant activity was decreased markedly.

Among the 21 analogues of valproic acid tested, valpramide, the primary amide of valproic acid, proved to be the most potent compound, being considerably more active than valproic acid in both threshold and (supra)maximal seizure tests. Valpramide has been shown to be also more potent than valproic acid at suppressing seizures induced by the GABA-antagonists, bicuculline and picrotoxin (Worms and Lloyd, 1981). However, as shown by the present experiments, valpramide was considerably more sedative and toxic than valproic acid after intraperitoneal injection in mice, which led to low protective indices. In regard to human epilepsy, valpramide has been marketed in some countries as an antiepileptic drug for oral administration. Utilizing

this route in humans, valpramide seems to be subject to degradation to valproic acid before reaching the systemic circulation, and only traces of unmodified amide have been determined in the plasma of patients on chronic therapy (Pisani, Fazio, Oteri and Di Perri, 1981). Being a pro-drug for valproic acid in man, valpramide administration seems to have advantages over treatment with valproic acid because of lower diurnal fluctuations in plasma levels of valproic acid (Pisani *et al.*, 1981).

Of the other close analogues of valproic acid tested, i.e. drugs with branching in position 2, compounds with shorter side-chains than valproic acid were only weakly active, whereas drugs with longer side-chains, i.e. 2-propylhexanoic acid, 2-butylhexanoic acid, 2-pentylheptanoic acid and 2-hexyloctanoic acid, exerted a greater anticonvulsant potency but were also more sedative and toxic than valproic acid. Similar observations have recently been reported for some of these compounds by Keane *et al.* (1983). Determination of active brain concentrations of valproic acid and close analogues of valproic acid in mice suggests that the increase in activity with elongation of side-chains is not a function of increased penetration into the brain due to enhanced liposolubility as suggested by Keane *et al.* (1983), but is due to a greater intrinsic activity of these drugs (Kochen, Tauscher, Klemens and Löscher, unpublished).

Branching in position 3 instead of position 2 was associated with a marked enhancement of anticonvulsant potency as shown by 3-propylhexanoic acid. This compound has previously been reported to be more potent than valproic acid in protecting mice against mortality produced by pentylenetetrazol (Taillandier *et al.*, 1975). However, the present experiments showed that the toxicity of 3-propylhexanoic acid was much greater than that of valproic acid.

Introduction of additional branchings with methyl groups in position 3 (di-isopropylacetic acid and 2-butyl-3-methylpentanoic acid) gave only moderate increases in anticonvulsant activity compared to the corresponding compounds with two branchings. In contrast, additional branching in position 2 of the molecule (2-ethyl-2-methylpentanoic acid and 2,2-dimethylpentanoic acid) resulted in marked potency elevation. However, as reported previously (Lespagnol *et al.*, 1972; Maitre *et al.*, 1974), especially in the case of 2-ethyl-2-methylpentanoic acid a considerable enhancement of sedative/hypnotic adverse effects compared to valproic acid was found. The latter compound proved also to be about 3 times more toxic than valproic acid, both after intraperitoneal and intravenous injections.

In any event, a branched chain was necessary for anticonvulsant action as both non-branched monocarboxylic acids, i.e. octanoic acid and 4-pentenoic acid, and compounds in which the side-chains were closed to a ring, i.e. cyclopentanoic, cyclohexanoic and cycloheptanoic acid, were devoid of anticonvulsant action or only weakly active. Consistent with the present data, absence of anticonvulsant activity has also been reported for butyric, pentanoic and hexanoic acid (Keane *et al.*, 1983). Although non-branched fatty acids are not anticonvulsants, they produce sedation which increases as a function of chain length (Samson, Dahl and Dahl, 1956). In line with branching as a prerequisite of anticonvulsant action, addition of a methyl group in position 1 at the ring of cyclohexanoic acid resulted in an 8-fold increase of anticonvulsant potency, but the respective compound (1-methyl-1-cyclohexanoic acid) was also clearly more sedative than valproic acid.

In conclusion, from the present experiments it appears that within a large series of branched monocarboxylic acids valproic acid has the optimal chemical structure in regard to margins between anticonvulsant effects and sedative/hypnotic side effects. It should be noted that ethosuximide, one of the major antiepileptic drugs used against "absence" seizures in humans, was superior to valproic acid as regards anticonvulsant potency against pentylenetetrazol-induced seizures as well as protective indices and margins of safety by this seizure test. On the other hand, in the maximal electroshock test, ethosuximide was inferior to valproic acid and all metabolites and analogues tested in this model for *grand mal* epilepsy. Although the present study did not yield new drugs that were more active and less sedative than valproic acid, teratological studies suggest that some of the analogues of valproic acid may have advantages in regard to teratogenicity. Thus, as 2-en-valproic acid, compounds with an additional branching with a methyl group at the carbon next to the carboxyl group (2,2-dimethylpentanoic acid, 2-ethyl-2-methylpentanoic acid and 1-methyl-1-cyclohexanoic acid) lack the teratogenic potential of valproic acid (Nau and Löscher, unpublished). With respect to these findings and the present data on these compounds, 1-methyl-1-cyclohexanoic acid and 2,2-dimethylpentanoic acid seem to be potentially interesting drugs, because both have anticonvulsant ED_{50}s and LD_{50}s which are very similar to the respective values of valproic acid. Although these two drugs are more sedative than valproic acid in mice, their anticonvulsant effectiveness should be studied in more sensitive models, e.g. in genetic animal models of epilepsy (cf. Löscher and Meldrum, 1984), in order to see if the drugs are also active at non-sedative doses.

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